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(71) Applicant *(for all designated States except US)*: AKZO NOBEL N.V. [NL/NL]; Velperweg 76, NL-6824 BM Arnhem (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KELDERMANS, Cornelia, Elisabeth, Johanna, Maria [NL/NL]; Jacob Tilstraat 15, NL-1067 PN Amsterdam (NL). HORZINEK, Marian, Christian [NL/NL]; Haydnlaan 15, NL-3723 KE Bilthoven (NL). DE RONDE, Anthony [NL/NL]; Wethouder Tabakstraat 100, NL-1107 DE Amsterdam (NL). EGBERINK, Hermanus, Franciscus [NL/NL]; Prof. Dr. Bakkerlaan 57, NL-3431 EH Nieuwegein (NL).

(74) Agent: MESTROM, J., J., L.; Postbus 20, NL-5340 BH Oss (NL).

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(54) Title: POLYPEPTIDE FRAGMENT CAPABLE OF INDUCING NEUTRALISING ANTIBODIES AGAINST FELINE IMMUNO-DEFICIENCY VIRUS

(57) Abstract

The present invention relates to polypeptides of the Feline Immuno-deficiency virus surface protein, capable of inducing neutralising antibodies against FIV. The invention also relates to a neutralising monoclonal antibody recognising a region on the FIV surface protein, recognizing an epitope at polypeptides capable of inducing neutralising antibodies. A vaccine against FIV is also part of the invention.

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Polypeptide fragment capable of inducing neutralising antibodies against Feline Immuno-deficiency virus.

The present invention is concerned with a polypeptide fragment of the Feline Immuno-deficiency virus surface protein, immunogens comprising the polypeptide fragment, a nucleic acid sequence encoding the polypeptide fragment, a recombinant nucleic acid molecule containing the nucleic acid sequence, virus vectors containing the nucleic acid sequence or the recombinant nucleic acid molecule, host cells containing that nucleic acid sequence or the recombinant nucleic acid molecules or the vector virus, a vaccine for the protection of cats against Feline Immuno-deficiency virus, monoclonal antibodies reactive with the polypeptide fragment, and the use of the polypeptide or the immunogen for the preparation of a vaccine against Feline Immuno-deficiency virus.

The Feline Immuno-deficiency virus (FIV) is a recently discovered T-lymphotropic lentivirus, initially isolated from an immuno-deficient cat in 1986 in the United States (Pedersen et al, Science 235: 790-793 (1987)).

FIV-infection in cats may lead to immunological abnormalities similar to those seen in Human Immuno-deficiency virus type 1 (HIV-1) infected humans, like a depletion of $CD4^+$ cells in the circulation. (Ackley et al, J. Virol. 64: 5652-5655 (1990), Barlough et al, J. Acquired Immune Defic. Syndr. 4: 219-227 (1991), Torten et al, J. Virol. 65: 2225-2230 (1991)). Similarly, the peripheral blood mononuclear cells

(PBMC) from FIV-infected cats show reduced proliferative responses to mitogens and to exogenous interleukin 2 in vitro (Torten et al, J. Virol. 65: 2225-2230 (1991), Hara et al, Jpn. J. Vet. Sci. 52: 573-579 (1990), Siebelink et al, AIDS Res. Hum. Retroviruses 6: 189-196 (1990)).

The pathogenesis is much alike HIV-1 pathogenesis: cats, experimentally infected with FIV appear normal for about 4-6 weeks. At that time they develop a low-grade fever, neutropenia and mild leukopenia, and generalised lymphadenopathy. This lymphadenopathy may persist up to 9 months. After this period, most animals are completely recovered from initial infection. After one year or more after initial infection, the onset of the terminal AIDS-like phase may take place.

As is the case with human HIV-1 infection, in most cases opportunistic infections lead to the death of the infected cats.

Lentiviruses by nature do display a large degree of molecular and biological variation. This natural variation is generally ascribed to the low fidelity of the viral enzyme reverse transcriptase in the process of copying the viral genomic RNA to DNA (Preston et al, Science 242: 1168-1171 (1988), Roberts et al, Science 242: 1171-1173 (1988)). As a result, several variant FIV-strains have been found.

To date, isolates of several variant FIV strains, some of which have been subjected to molecular cloning, have been described. Amongst these strains are two isolates from the United States (Petaluma-strains (Olmsted et al, Proc. Natl. Acad. Sci USA 86: 2448-2452 (1989), Talbott et al, Proc. Natl. Acad. Sci. USA 86: 5743-5747 (1989)) and San Diego strain (Phillips et al, J. Virol. 64: 4605-4613 (1990))), one from the United Kingdom (Harbour et al, Vet. Rec. 122: 84-86 (1988)) and two from Japan (Ishida et al, J. Am.

Vet. Med. Assoc. 194: 221-225 (1989), Miyazawa et al, Arch. Virol. 108: 59-68 (1989)), which were obtained from the DNA of in vitro propagated strains.

Molecular characterisation and determination of heterogeneity between FIV isolates has been described by Maki et al (Arch. Virol. 123: 29-45 (1992)). The construction of DNA clones from two FIV proteins, i.e. the Envelope protein and the Gag protein and their use for detecting and preventing FIV has been described in WO 92/15684.

Sero-epidemiological surveys have revealed, that the virus has spread all over the world (Furuya et al, Jpn. J. Vet. Sci. 52: 891-893 (1990), Gruffydd-Jones et al, Vet. Rec. 123: 569-570, (1988), Ishida et al, Jpn. J. Vet. Sci. 52: 453-454 (1990), Ishida et al, Jpn. J. Vet. Sci. 50: 39-44 (1988), Ishida et al, J. AM. Vet. Med. Assoc. 194: 221-225 (1989), Swinney et al, N.Z. Vet. J. 37: 41-43 (1989)).

Vaccination against FIV with inactivated vaccines so far has been shown to be effective only for one specific strain; the Petaluma strain. Inactivated whole cell preparations and inactivated whole virus preparations were used in this experiment. (Yamamoto et al; J. of Virol. 67: 601-605 (1993)). Identical experiments done with the Glasgow strain by Hosie et al (Proceed of the First Int. Conf. Of FIV Researchers, Univ. of Cal. Davies, p. 64 (1991)) and Jarrett et al (AIDS 5 (Suppl.) S.163-S.165 (1991)) did not lead to protection. On the contrary, it led to immune-enhancement. The mechanism behind this phenomenon is unclear, but certainly unwanted.

The use of subunit-based vaccines as described in the present application has a number of significant advantages over the use of whole virus vaccines:

a) There is no need for culturing live virus. This eliminates the introduction of unwanted mutations leading to more immunologically variant strains during virus growth. The occurrence of mutations in RNA-viruses is known to be high compared to DNA-viruses. For retroviruses, like e.g. FIV, the mutation rate is even higher, due to the high error rate of reverse transcriptase. The occurrence of escape mutants in vivo, (viruses, not recognized by their host's defenses), and the role of antibody escape in viral persistance have recently been described by Pancino et al (Journ. of Virol 67: 664-672 (1993). In this paper, several regions, i.a. the region between amino acids 365-424 are shown to be immunogenic, and therefore they are proposed as regions for use in diagnostic tools.

b) There is obviously no need for inactivation of live virus. In cases where live virus is used, checking for full inactivation has to be done extremely careful and therefore is laborious, time-consuming and costly, as is shown by Yamamoto et al.(J. of Virol. 67: 601-605 (1993)).

c) By using short fragments instead of whole virus preparations, the risk of raising unwanted antibodies against epitopes involved in immune enhancement is significantly reduced.

Additionally, the use of live attenuated virus poses the problem of how to create a sufficiently attenuated vaccine, especially for the following reason: infected cats, especially in a later stage of infection may be immuno-impaired, and as a result, would suffer from severe illness, due to vaccination with even a highly attenuated live vaccine. (Gardner et al, Veterinary Medicine vol. march; 300-307. (1991)).

Aiming at the subunit approach, research efforts are mainly aimed at the localisation of the immunologically important immunogenic determinants, the so-called epitopes at the FIV proteins.

In order to precisely locate these epitopes, usually short polypeptides, synthesized either chemically or in prokaryotic expression systems are used.

This kind of approach, due to the nature of the techniques used, has several major drawbacks:

a) most epitopes, including neutralising epitopes are conformational (i.e. depending on the 3-dimensional structure of the protein) discontinuous or even discontinuous scattered epitopes. Due to the way they were synthesized, they will usually not be present in their native form and they are therefore in many cases not representative for the immunogenic properties of the native epitope. (Snijders et al, J. Gen. Virol 72: 557-566 (1991), Gebauer et al, Virology 183: 225-238 (1991)). A striking example is given in the case of Simian Immuno-deficiency virus and HIV-2, Here it was shown, that linear epitopes corresponding to the V3 loop in the surface protein, in contrast to conformational epitopes, do not elicit neutralising antibodies (Javaherian et al; Proc. Natl. Acad. Sci. USA 89: 1418-1422 (1992)).

b) the approach predominantly leads to the detection of immuno-dominant regions on proteins.

c) in many cases, the immuno-dominant regions do not coincide with neutralising epitopes. This lack of correlation between immuno-dominant regions and neutralising epitopes has been demonstrated for many viruses, e.g. for HIV, where antibodies against gp41 immuno-dominant regions have extensively been described, although never any antibodies against this region have been found to display anti-viral activity. (Viscidi et al, AIDS-Res-Hum-Retroviruses 6:

1251-1256 (1990), Bugge et al, J. Virol. 64: 4123-4129 (1990), Teeuwsen et al, AIDS-Res-Hum-Retroviruses 6: 381-392 (1990)). On the contrary, antibodies against these regions have been shown to enhance infectivity (so-called immune-enhancement). (Robinson et al, Proc. Natl. Acad. Sci USA 87: 3185-3189 (1990), Robinson et al, J. Virol. 64: 5301-5305 (1990)).

This is also applicable for non-retroviruses. For example, for Duck Hepatitis virus, it was shown, that antibody response to neutralising epitopes is weak or non-existent, whereas immuno-dominant regions do elicit a firm but non-protective immune response. (Cheung et al, Virology 176: 546-552 (1990)).

Given the fact that only few out of many antigenic determinants are neutralising determinants, and given the fact that, as explained above, by using techniques well-known in the art, nonlinear epitopes are generally not detected, until now only immuno-dominant epitopes on the FIV-surface protein have been found, as was to be expected, and described i.a. by Avrameas et al (Molecular Immunology; 25/5: 565-572 (1992)) and International Patent Application WO 9209632-A1.

Until now, no neutralising epitopes have been found on the surface protein of FIV.

Protection against infection or against the consequences of infection however can (apart from cellular immunity) only be efficient, if neutralising antibodies are induced.

Surprisingly a polypeptide fragment of the Feline Immuno-deficiency virus surface protein, capable of inducing neutralising antibodies against FIV has been identified now.

Furthermore, a conformational epitope, reactive with a neutralising monoclonal antibody has been located.

Therefore, the present invention provides a polypeptide fragment of the Feline Immuno-deficiency Virus surface protein, characterised in that the polypeptide fragment comprises an amino acid sequence given in SEQ ID NO: 4 or a portion thereof capable of inducing neutralising antibodies against Feline Immuno-deficiency Virus. The fragment given in SEQ ID NO: 4 may also be referred to as the Central Fragment. The fragment in SEQ ID NO: 4 is a part of the FIV-protein shown in SEQ ID NO: 2.

The present invention also provides a polypeptide fragment of the Feline Immuno-deficiency Virus surface protein, characterised in that the polypeptide fragment comprises an epitope located in the amino acid sequence given in SEQ ID NO: 4, capable of inducing antibodies that competitively inhibit binding of the neutralising monoclonal antibody from hybridoma 1E1EB4-93030567 as deposited with the European Collection of Animal Cell Cultures (further referred to as the ECACC), Division of Biology. Salisbury, Wiltshire, SP4 OJG, United Kingdom, to native surface protein.

The term "polypeptide fragment" refers to an amino acid sub-set of the amino acid sequence representing the surface protein, comprising amino acids 361 to 445, the Central Fragment, or a portion thereof, and described in SEQ ID NO: 4. The term "portion" here refers to a subset of the amino acids represented by the SEQ ID NO: 4.

As mentioned above, several variant strains of FIV have been determined. In general, these variants will have minor differences in the amino acid sequence of their respective surface proteins. This is due to natural variation in the nucleic acid sequence coding for the respective surface proteins. In all cases, the result of these variations is a biologically functional surface protein. Functional equivalence can be expressed in a clear mathematical form, due to the algorithm developed by Lipman et Pearson (Science 227: 1435-1441 (1985) for comparison of variant proteins.

Variation may be the result of insertion or deletion of one or more amino acids, or of replacement of one or more amino acids by functional equivalents. Replacement by functionally equivalents is often seen. Examples described by Neurath et al (The Proteins, Academic Press, New York (1979), page 14, figure 6) are i.a. the replacement of the amino acid alanine by serine; Ala/Ser, or Val/Ile, Asp/Glu, etc. In addition to the variations mentioned above, variations have been found, in which an amino acid has been replaced by another amino acid that is not a functional equivalent. This kind of variation only differs from replacement with functional equivalents in that it may yield a protein that has a slight modification in its spacial folding.

Therefore, variations in the nucleic acid sequence coding for the surface protein, leading to variations in the amino acid sequence of the surface protein but leaving the protein immunologically active are also within the scope of the present invention.

The term "epitope" refers to an amino acid sequence containing at least 8 amino acid sequences, and capable of inducing (with or without flanking amino acids or immunostimulatory compounds) an immunological reaction in a suitable host animal

(Geysen, et al; Proc. Natl. Acad. Sci. USA 81: 3998-4002 (1984)).

As has also been explained above, an epitope may also span a polypeptide fragment larger than 8 amino acids, also depending on the epitope's conformational nature. Thus, "epitope" in this context may refer to any amino acid sequence equal to, or larger than 8 amino acids.

The expression "immunogenic" refers to amino acid sequences capable of triggering the immune system.

The expression "immuno-dominant region" refers to an amino acid sequence that is capable of inducing a more than significant antibody response. This induction may result in relatively large amounts of antibodies directed against one single epitope, or in antibodies directed against a number of epitopes within this region.

"Neutralizing antibodies" are antibodies capable of preventing the virus from multiplication in the host, thus interfering with the process of pathogenesis in such a manner, that the process of pathogenesis is inhibited. The role of neutralising antibodies has i.a. been described extensively by Fazekas de St. Groth (The neutralisation of viruses; Advances in Virus Research 9: 1-125 (1962))

It is not always necessary, and also not always desirable to use a large polypeptide for the induction of antibodies. Large polypeptide fragments flanking the immunologically important region may represent an unnecessary high antigenic load, resulting in a less efficient or non-specific triggering of the immune system.

Thus one may decide to remove natural flanking sequences from the immunologically preferred region of a polypeptide. This can be done e.g. with the use of protein-digesting enzymes, e.g. proteinase K and V8-protease.

It is also possible to use the available amino acid information to synthesize the desired polypeptide fragment by using chemical synthesis. In that case, every unwanted amino acid sequence can be deliberately left out. One often used method for the chemical synthesis of short polypeptides is the Merrifield synthesis (Merrifield et al; J. Am. Chem. Soc. 85:2149 (1963)). Another way of synthesizing the polypeptide fragment is to clone the rDNA coding for the polypeptide into an expression vector and to express the genetic information in a suitable expression system. This possibility is described in detail below.

Based on the above mentioned, the polypeptide fragment in a preferred form is a portion of the central fragment and said portion comprises at least an epitope located in between amino acid 389 and amino acid 412, or an epitope reactive with monoclonal antibody from hybridoma 1E1EB4-93030567 deposited with the ECACC.

In an even more preferred embodiment, the portion of the polypeptide fragment is selected from the group of sequences comprising SEQ ID NO: 5, 6, 7.

The invention also relates to an immunogen comprising a polypeptide fragment according to the present invention, linked to a carrier.

Generally, the word "carrier" applies to molecules that are covalently linked to a polypeptide fragment of the invention and as such "carry" the polypeptide fragment.

The word "immunogen" here refers to a polypeptide fragment of the present invention, presented to the immune system of a suitable host in such a form that it is capable of inducing an immunological response.

It is well known to those skilled in the art, that the immunogenicity of polypeptides may be significantly enhanced by adding, or linking other molecules to a polypeptide of the present invention.

In addition, very short polypeptides, e.g. polypeptides with a length of 8 amino acids, are not immunogenic as such.

Therefore, these short polypeptides may be linked in various ways to other molecules, so-called carrier-molecules. These carrier-molecules may e.g. be polypeptides.

One way of making such an immunogen is the use of chemical methods to link a polypeptide fragment of the present invention to a carrier-protein. Proteins often used as carriers are e.g. Keyhole Limpet Haemocyanine and Bovine Serum Albumin. Methods of chemical linkage of polypeptides are i.a. described by Reichlin, M.; Methods in Enzymology 70: 159-165 (1980) and by Erlanger, B.F.; Methods in Enzymology 70: 85-103 (1980).

Another way of making such an immunogen is the molecular cloning of the nucleotide sequence coding for a polypeptide of the present invention upstream, downstream or in between nucleotide sequences coding for another protein. Expression of this construct will then lead to a larger polypeptide, in which the polypeptide of the present invention is preceded, flanked or followed by other polypeptide sequences. Suitable flanking sequences could be those, coding for KLH or BSA, but many other protein sequences would be applicable as well.

Another suitable group of carrier molecules is the group comprising the complex carbohydrates. It is possible to chemically link a polypeptide of the present invention to a carbohydrate with the aim of

enhancing the immuno-reactivity of the thus formed complex. Methods for covalent linkage of polypeptides to carbohydrates have been described a.o. by Dick, W.E. and Beurt, M.; Contrib. Microbiol. Immunol. 10:48-114 (1989).

It is clear, that other carrier types or other methods of linkage of a polypeptide to a carrier are also embodied in the present invention.

Therefore, in a preferred form, the carrier is selected from the group of carriers consisting of surface-active compounds, sugars and proteins.

The invention also provides a nucleic acid sequence encoding a polypeptide fragment or the immunogen according to the present invention.

In principle, the amino acid building blocks of the polypeptide each have a corresponding nucleic acid triplet coding for that specific amino acid. This does not mean, however, that a single amino acid also has one single nucleic acid triplet coding for it. On the contrary, most amino acids have two to even six (Leucine) possible coding nucleic acid triplets. This phenomenon is known as the degeneracy of the genetic code.

It goes without saying that, as a result of this phenomenon, the scope of the invention extends to all nucleic acid sequences encoding a polypeptide fragment of the present invention.

In addition to this, and as has been explained above, variations in nucleic acid sequence leading to different but functionally homologous amino acids (functional replacement, e.g. replacement of Alanine by Serine) are also considered to be within the scope of this invention.

In a preferred embodiment, said nucleic acid sequence comprises at least part of the nucleic acid sequence shown in SEQ ID NO: 3.

In a further embodiment of the present invention, said nucleic acid sequence is part of a recombinant nucleic acid molecule comprising the nucleic acid sequence under the control of regulating sequences enabling expression of the protein encoded by said nucleic acid sequence.

Regulating sequences enabling expression of genes or fragments of genes may e.g. be promotor-sequences either or not in combination with enhancer sequences. Promotor sites are sequences to which RNA polymerase binds, initial to transcription.

Promotor-sites exist in a variety of types, a.o. depending on the type of cell, they originate from. Promotor sequences have been described for promoters from prokaryotic, eukaryotic, and viral origin.

Recombinant DNA molecules of the above mentioned type can be made by cutting a suitable DNA fragment with a suitable restriction enzyme, cutting a fragment containing regulating sequences with the same enzyme and ligating both fragments in such a way, that the nucleic acid sequence to be expressed is under the control of the promotor sequence. Many variant approaches to make useful recombinants have been described in Sambrook (Sambrook et al, Molecular cloning, a laboratory manual. Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989)).

In general, recombinant nucleic acid sequences will be cloned into a vector molecule. The then formed recombinant vector molecule, often capable of self-replication in a suitable host cell, can be used to bring the cloned nucleic acid sequences into a cell. This may be a cell in which replication of the recombinant vector molecule occurs. It may also be a

cell in which a regulating sequence of the vector is recognised, so that a polypeptide fragment according to the present invention is expressed.

A wide range of vectors is currently known, including vectors for use in bacteria, e.g. pBR322, 325 and 328, various pUC-vectors a.o. pUC 8, 9, 18, 19, specific expression-vectors; pGEM, pGEX, and Bluescript(R), vectors based on bacteriophages; lambda-gtWes, Charon 28, M13-derived phages, vectors containing viral sequences on the basis of SV40, papilloma-virus, adenovirus or polyomavirus (Rodriquez, R.L. and Denhardt, D.T., ed.; Vectors: A survey of molecular cloning vectors and their uses, Butterworths (1988), Lenstra et al, Arch. Virol.; 110: 1-24 (1990)).

All recombinant molecules comprising the nucleic acid sequence under the control of regulating sequences enabling expression of the protein encoded by said nucleic acid sequence are considered to be part of the present invention.

The nucleic acid sequence coding for a polypeptide, according to the present invention may be cloned either or not under the control of a promotor sequence, in a viral genome. In this case, the virus may be used as a way of transporting the nucleic acid sequence into a target cell. Such recombinant viruses are called vector viruses. The site of integration may be a site in a gene, not essential to the virus, or a site in an intergenic region. Viruses often used as vectors are Vaccinia viruses (Panicali et al; Proc. Natl. Acad. Sci. USA, 79: 4927 (1982), Herpesviruses (E.P.A. 0473210A2), Retroviruses (Valerio, D. et al; in Baum, S.J., Dicke, K.A., Lotzova, E. and Pluznik, D.H. (Eds.), Experimental Haematology today - 1988. Springer Verlag, New York:pp. 92-99 (1989)) and

baculoviruses (Luckow et al; Bio-technology 6: 47-55 (1988).

The invention also comprises a virus vector containing a nucleic acid sequence encoding the polypeptide fragment, or a recombinant nucleic acid molecule encoding the polypeptide fragment under the control of regulating sequences enabling expression of the protein encoded by said nucleic acid sequence.

Furthermore the invention comprises a host cell containing a nucleic acid sequence encoding the polypeptide fragment, or a recombinant nucleic acid molecule encoding the polypeptide fragment under the control of regulating sequences enabling expression of the protein encoded by said nucleic acid sequence.

The invention also comprises a host cell containing a virus vector containing a nucleic acid molecule encoding the polypeptide fragment, or a recombinant nucleic acid molecule encoding the polypeptide fragment under the control of regulating sequences enabling expression of the protein encoded by said nucleic acid sequence.

A host cell may be a cell of bacterial origin, e.g. Escherichia coli, Bacillus subtilus and Lactobacillus species, in combination with bacteria-based vectors as pBR322, or bacterial expression vectors as pGEX, or with bacteriophages. The host cell may also be of eukaryotic origin, e.g. yeast-cells in combination with yeast-specific vector molecules, or higher eukaryotic cells like insect cells (Luckow et al; Bio-technology 6: 47-55 (1988)) in combination with vectors or recombinant baculoviruses, plant cells in combination with e.g. Ti-plasmid based vectors or plant viral vectors (Barton, K.A. et al; Cell 32: 1033 (1983), mammalian cells like Hela cells, Chinese Hamster Ovary cells (CHO) or Crandell Feline Kidney-

cells, also with appropriate vectors or recombinant viruses.

Based on the polypeptide fragment of the invention, a vaccine for the protection of cats against Feline Immuno-deficiency Virus infections can be made.

The vaccine may comprise said nucleic acid sequence or a recombinant nucleic acid molecule as explained above or said vector virus or said host cell.

The vaccine may also comprise the polypeptide fragment mentioned before or the immunogen mentioned above.

In a preferred presentation, the vaccine also comprises an adjuvant. Adjuvants in general comprise substances that boost the immune response of the host in a non-specific manner. A number of different adjuvants are known in the art. Examples of adjuvants are Freunds Complete and Incomplete adjuvant, vitamin E, non-ionic block polymers, muramyldipeptides, Quill A(R), mineral oil e.g. Bayol(R) or Markol(R), vegetable oil, and Carbopol(R) (a homopolymer).

The vaccine may also comprise a so-called "vehicle". A vehicle is a compound, or to which the polypeptide adheres, without being covalently bound to it. Often used vehicle compounds are e.g. aluminium hydroxide, -phosphate or -oxide, silica, Kaolin, and Bentonite

In addition, the vaccine may comprise one or more suitable surface-active compounds or emulsifiers, e.g. Span or Tween.

It goes without saying, that other ways of adjuvating, adding vehicle compounds or emulsifying a polypeptide are also embodied in the present invention.

One way of interfering with the process of pathogenesis, is passive immunization with antibodies. These antibodies when administered to the host may interfere with the invading virus in such a way as to prevent pathogenesis. Antibodies can be made in a number of ways. One very often used method is vaccination of horse, goat, rabbit etc. and collecting serum after antibody response has been detected. This method yields a variety of antigens that react with the polypeptide used for immunization.

Another method for obtaining antibodies is the method for making so-called monoclonal antibodies. It depends on the production and selection of one specific antibody type reactive with one specific epitope. The method for production of monoclonal antibodies by using the hybridoma technique has been published a.o. by Kohler and Milstein (Nature 256: 459 (1975)), Kohler and Milstein (Eur. J. Immunol. 6: 511 (1976)), Gefter et al (Somatic Cell Genet. 3: 231 (1977)), Volk et al (J. Virol. 42: 220 (1982)) and Hammerling et al (Monoclonal Antibodies and T-Cell Hybridomas, Elsevier New York, pp. 563-681 (1981)).

In the present invention, using mainly the methods cited above, mouse monoclonal antibodies were made that were shown to be reactive with an epitope, located on the Central Fragment of the FIV surface protein.

In brief, Swiss outbred mice were vaccinated twice with sufficiently large doses of an inactivated whole virus preparation, in order to obtain a clear anti-FIV antibody response. Fusions were made after antibody response was reached, between myeloma cells and mouse spleen cells. Hybridomas were tested for antibody production, and among positive clones, i.a. a hybridoma was found to produce a neutralising epitope

recognising a conformational epitope of the FIV surface protein located in the Central Fragment.

The invention thus relates to monoclonal antibodies that are reactive with the polypeptide as described in SEQ ID NO: 4 or a portion thereof, or immunologically active variants thereof.

In a more preferred form, the monoclonal antibody is from the hybridoma 1E1EB4-93030567 deposited with the ECACC.

The present invention also relates to the use of the polypeptide or the immunogen for the preparation of a vaccine for the prophylaxis of Feline Immunodeficiency Virus infection.

Example I

Isolation of genomic DNA of FIV-infected cells and sequencing.

Genomic DNA of FIV-113 infected cells was isolated and digested to completion with NheI. Fragments hybridizing with both the Pol-gene and the U3-R region of the FIV-LTR, and thus comprising the genetic information for the surface protein, were used for further subcloning and subsequently sequenced. All DNA-techniques were carried out essentially as described by Sambrook (Sambrook et al, Molecular cloning, a laboratory manual. Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989)).

The sequence comprising the FIV-surface protein code is given in SEQ ID NO: 1.

The pOTSKF33 plasmid vector (Chiang et al, Clin. Chem. 35: 946-952 (1989), Krone et al, J. Med. Virol 26: 261-270 (1988)) encoding the amino-terminal part of galactokinase (galK) controlled by an inducible promoter was used to construct fusion proteins between galactokinase and the surface protein of FIV strain UT113. Standard cloning techniques (Sambrook et al, Molecular cloning, a laboratory manual. Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989)) using convenient restriction enzyme sites within the FIV-UT113 surface protein coding sequence were applied to obtain the galK-CF in frame fusion construct containing the central fragment (further referred to as CF) spanning amino acids 361 - 445. The galK-CT fusion encoding the carboxyl-part of the surface protein (further referred to as CT) spanning amino acids 516 - 611 was constructed using PCR with the carboxyl end at the cleavage site between the surface and transmembrane (TM) protein. The galK-CTδT fusion is identical to galK-CT with a deletion from amino acid 599 to 611.

The localisation of the fragments CF, CT and CTδT is depicted in figure 1.

The expression and purification of galK-CF, galK-CT and galk-CTδT fusion proteins was performed as described (Aldovini et al, Proc. Natl. Acad. Sci USA 83: 6672-6676 (1987), Krone et al, J. Med. Virol 26: 261-270 (1988)). Briefly, fusion protein expression was induced by adding 60 µg/ml nalidixic acid to exponential growing cultures of transformed AR120 bacteria. Four hours after induction of expression bacteria were lysed by sonification and protein was purified as described (Krone et al, J. Med. Virol 26: 261-270 (1988)) using preparative SDS-PAGE and finally electro-elution of the purified fusion protein. Purity of the fusion protein was checked on SDS-PAGE gels by Coomassie blue staining and by immuno-blotting using

an antiserum directed against the galK common part of the fusion proteins.

Example II

Antibodies against CF, CT and CTδT proteins in cat sera raised against whole FIV.

The galK-CF, galK-CT and galk-CTδT fusion proteins were used to develop an ELISA for the detection of surface specific antibodies in sera of FIV-infected cats. Sera of cats prior to infection with FIV did not show FIV CF, CT and CTδT specific antibodies indicating the specificity of the ELISA (table 1). All cats showed a seroconversion for antibodies to at least one of the CF and CT proteins. The seroconversion occurred starting from week 6 after FIV infection depending on the isolate and dose of inoculation used. All cats showed antibodies to CF, albeit that the levels of antibodies showed some variation. The second best recognized protein was CT against which in 15 out of 24 cat sera antibodies could be detected.

The results of CTδT are indicative for the fact that certainly not all Surface protein fragments are immunogenic. In this case is was shown that an epitopes is located within the last 13 C-terminal amino acids.

The surface fragments CF and CT being the most immuno-dominant ones were also the ones most frequently recognized early after infection.

ELISA

Ninety six well plates (Greiner, high bond) were coated overnight at 4 °C with the galK-CF or galk-CT fusion protein (100 ng per well in PBS). To exclude a different coating efficacy for the different fusion proteins, coating efficacy was checked with a rabbit serum (anti-NEF, (De Ronde et al, Virol. 188: 391-395 (1989))) directed against the galK common part of all fusion proteins. Residual protein was removed by a wash procedure consisting of three washes with PBS; 0.05% Tween-20 and two washes with PBS. To block non-specific binding of proteins to the plates in subsequent steps of the ELISA procedure the plates were incubated with PBS; 0.05% Tween-20; 5% goat serum at 37 °C for 1 hour. Blocking was followed by a wash procedure (see above). Sera diluted in PBS; 0.05% Tween-20; 5% goat serum (routinely 1:100 for cat) were incubated at 37 °C for one hour. After a wash procedure (see above) the plates were incubated with an horse radish peroxidase (HRP) labelled goat-anti-cat-serum (Cappel, routinely diluted 1/9000 in PBS; 0.05% Tween-20; 5% goat serum) at 37 °C for one hour. Horse radish peroxidase activity was detected by incubation with H_2O_2/Tetramethylbenzidine (Sigma). The reaction was stopped by addition of 2M H_2SO_4 and was standardized against a series of dilutions of a known positive cat serum. Optical density of the samples was determined at 450 nm.

Example III

Immunization of rabbits

Rabbits (New Zealand white) were injected subcutaneously with 100 µg of the galK-CF or galk-CT fusion protein in Freunds complete adjuvant. Every three weeks the rabbits were boosted with 100 µg of the galK-CF or galk-CT fusion protein in Freunds incomplete adjuvant. Hyperimmune sera reacted on immuno-blots with the FIV surface protein as produced in CRFK cells and in a baculo virus based expression system.

Immunization of cats

Outbred cats were injected subcutaneously with 100 µg of the galK-CF protein in an oil/alum adjuvant supplemented with G-MDP. Every six weeks the cats received a booster injection. Hyperimmune sera reacted with the FIV surface protein as produced in a baculo virus based expression system.

Example IV

Neutralization by sera directed to distinct parts of CF in rabbits and cats

To identify a biological relevant role of antibodies against the distinct surface protein fragments, polyclonal sera against the fragments were raised in rabbits. These polyclonal rabbit sera were assayed for neutralizing activity (see below). The serum of rabbits immunized with fusion protein CF induced neutralizing titers comparable to those in naturally infected cat serum, whereas sera of rabbits immunized with other parts of the surface protein did not induce significant neutralizing titers. This indicates that the fusion protein CF contains one or more neutralizing epitopes.

To verify that the CF protein as such was antigenic in cats as well, it was injected in cats. After one booster injection the CF protein induced neutralizing antibodies in cats with titers somewhat lower than in the rabbits which received multiple booster injections. Results are given in table 2.

Neutralization assay

At day 1, CRFK cells (Crandell et al, In vitro 9: 176-185 (1973)) (3500/well) were seeded in an 96-well plate and maintained in DMEM supplemented with 5% fetal calf serum. At day 2, 50 $TCID_{50}$ of CRFK derived FIV-UT113 was incubated for 1 hour at 37 °C with serial dilutions of the serum to be assayed. CRFK cells were washed with PBS + DEAE (50 µg/ml) and were incubated with the virus/serum mixture. At day 3, CRFK cells were washed with PBS and subsequently propagated in DMEM supplemented with 2% fetal calf serum. At day 8, the supernatant of the CRFK cells was assayed for viral p24 gag production. An inhibition of p24 production greater than 90% was considered as neutralization.

Example V

Immunological scanning of sera with neutralizing activity

The neutralizing rabbit serum, a neutralizing monoclonal, a representative neutralizing cat serum, and control rabbit and cat sera were analysed with overlapping short polypeptides, together representing the whole surface amino acid sequence contained within the CF fusion protein. Both the cat serum and the rabbit serum recognized peptides with the core sequence WRPDFE (amino acids 402-407). In that region of the surface protein the cat serum recognized a wider spectrum of peptides including the WRPDFE core

sequence and apparently consisting of multiple core sequences encompassing the SWKQGNRWEWRPDFESERV stretch of amino acids (amino acids 393-411). Results of the scanning are given in figure 2.

The neutralising monoclonal antibody does not directly react with the CF protein, but is prevented from binding to the FIV surface protein by the polyclonal rabbit serum against the Central Fragment polypeptide, synthesized in bacteria. This indicates that a similar region of the surface protein is recognised by rabbit as well as mouse antibodies. It is also concluded, that the mouse monoclonal antibody is directed to a conformational epitope.

In conclusion, the CF region of the surface protein of FIV contains a neutralising domain of linear as well as conformational architecture capable of eliciting neutralising antibodies against FIV in cats.

Cat	Strain	Surface fragment		
		CF	CTδT	CT
14.1	UT-113	++++		++++
15.1		++++		++++
16.1		++++		
17.1		++++		+++
18.1		++++		++++
20.1		+++		++++
21.1		+++		
18.2		++		++
19.2		+		
Ko		+		++++
Bi		+++	++	++++
340	UT-Ktj	++++		+
342		++++		++++
352		++++		+
356		+++		
308		++++		
320		++	++++	++++
322		++++		
326		++++		++++
330		++++		+
336		+++		
831		++++		
833		++++		
199	Petaluma	++++	+	++++

Table 1

Antibody response against surface protein fragments in FIV-infected cats.

Sera of cats infected with different FIV isolates were screened by ELISA for antibodies against the surface protein fragments CF, CT and CTδT. The severity of the reaction of the cat sera against the fragments was expressed according to optical density values reflecting the level of the antibody response (+: OD= between cut off value and 0.4; ++: OD= 0.4-0.6; +++= 0.6-0.8; ++++= >0.9).

Serum	Reciprocal of neutralization titer
rabbit 2121 α-K-CF	320
rabbit 2195 α-K-CF	80
rabbit 1448 α-K-CTδT	<10
rabbit 2218 α-K-CT	<10
cat 6 α-K-CF	80
cat 8 α-L-CF	10
cat 9 α-K-CF	10-20
cat 10 control	<10
pool FIV-$^{+}$ cats	160-320

Table 2

Neutralization of cat and rabbit sera against envelope surface fragments.

Sera were tested in a neutralization assay. The reciprocal neutralization titers of pre-immune rabbit and cat sera were less than 10. Hyper-immune rabbit sera were derived from rabbits which received at least two booster injections. The cats received one booster injection. The pool of sera of FIV infected cats was derived from cats infected with FIV-Ktj (cats 320, 322, 326), and a reciprocal neutralizing titer which was relatively high amongst FIV infected cats tested so far.

Legend to the figures

Fig. 1

Map of envelope surface fragments.

Envelope surface fragments were constructed as described (materials and methods) using convenient restriction enzyme sites and primers for PCR.

Fig. 2

Peptide analysis of cat and rabbit sera.

Overlapping 12-mer peptides of CF (FIV surface protein amino acids 361-372, 362-373, etc. to 433-445) were synthesized on a solid support and serum antibodies were detected using ELISA. A: a pre-infection cat serum; B: a post infection serum of an FIV-infected cats (20.1, table 1) with a neutralizing reciprocal titer of 320; C: neutralizing rabbit serum (2121, table 2).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Akzo N.V.
(B) STREET: Velperweg 76
(C) CITY: Arnhem
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): 6824 BM
(G) TELEPHONE: 04120-66223
(H) TELEFAX: 04120-50592
(I) TELEX: 37503 akpha nl

(ii) TITLE OF INVENTION: Polypeptide fragment capable of inducing neutralising antibodies against Feline Immuno-deficiency virus.

(iii) NUMBER OF SEQUENCES: 7

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2571 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(B) STRAIN: FIV-113

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2571

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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ATG GCA GAA GGG TTT GTA GCC AAT GGA CAA TGG ATA GGA CCA GAA GAA    48
Met Ala Glu Gly Phe Val Ala Asn Gly Gln Trp Ile Gly Pro Glu Glu
 1               5                  10                  15

GCT GAA GAG TTA GTA GAT TTT GAA ATA GCA ACA CAA ATG AAT GAA GAA    96
Ala Glu Glu Leu Val Asp Phe Glu Ile Ala Thr Gln Met Asn Glu Glu
            20                  25                  30
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GGG CCA CTA AAT CCA GGA ATA AAC CCA TTT AGG GTA CCT GGA ATA ACA   144
Gly Pro Leu Asn Pro Gly Ile Asn Pro Phe Arg Val Pro Gly Ile Thr
         35                  40                  45

AAA CAA GAA AAG CAG GAA TAT TGT AGC ACA ATG CAA CCC AAA TTA CAA   192
Lys Gln Glu Lys Gln Glu Tyr Cys Ser Thr Met Gln Pro Lys Leu Gln
     50                  55                  60

GCT CTA AGG AAT GAA ATT CAA GAG GTA AAA CTG GAA GAA GGA AAT GCA   240
Ala Leu Arg Asn Glu Ile Gln Glu Val Lys Leu Glu Glu Gly Asn Ala
 65                  70                  75                  80

GGT AAG TTT AGA AGA GCA AGA TTT TTA AGA TAC TCT GAT GAA ACT ATA   288
Gly Lys Phe Arg Arg Ala Arg Phe Leu Arg Tyr Ser Asp Glu Thr Ile
                 85                  90                  95

TTG TCT CTG ATT TAC TTG TTC ATA GGA TAT TTT AGA TAT TTA GTA GAT   336
Leu Ser Leu Ile Tyr Leu Phe Ile Gly Tyr Phe Arg Tyr Leu Val Asp
            100                 105                 110

AGA AAA AGG TTT GGG TCC TTA AGA CAT GAC ATA GAT ATA GAA GCA CCT   384
Arg Lys Arg Phe Gly Ser Leu Arg His Asp Ile Asp Ile Glu Ala Pro
        115                 120                 125

CAA GAA GAG TGT TAT AAT AAT AAA GAG AAG GGT ATG ACT GAA AAT ATA   432
Gln Glu Glu Cys Tyr Asn Asn Lys Glu Lys Gly Met Thr Glu Asn Ile
    130                 135                 140

AAA TAT GGT AAA CGA TGC TTA GTA GGA ACA GCA GCT TTG TAC TTG ATT   480
Lys Tyr Gly Lys Arg Cys Leu Val Gly Thr Ala Ala Leu Tyr Leu Ile
145                 150                 155                 160

CTA GCT ATA GGA ATA ATA ATA ATA ATA CGG ACA ACC GAT GCT CAG GTA   528
Leu Ala Ile Gly Ile Ile Ile Ile Ile Arg Thr Thr Asp Ala Gln Val
                165                 170                 175

GTG TGG AGA CTT CCA CCA TTA GTA GTC CCA GTA GAA GAA TCA GAA ATA   576
Val Trp Arg Leu Pro Pro Leu Val Val Pro Val Glu Glu Ser Glu Ile
            180                 185                 190

ATT TTT TGG GAT TGT TGG GCA CCA GAG GAA CCC GCC TGT CAG GAC TTT   624
Ile Phe Trp Asp Cys Trp Ala Pro Glu Glu Pro Ala Cys Gln Asp Phe
        195                 200                 205

CTT GGG GCA ATG ATA CAT CTA AAA GCT AGT ACA AAT ATA AGT AAT ACA   672
Leu Gly Ala Met Ile His Leu Lys Ala Ser Thr Asn Ile Ser Asn Thr
    210                 215                 220

GAG GGA CCT ACC TTG GGG AAT TGG GCT AGA GAA ATA TGG GCA ACA TTA   720
Glu Gly Pro Thr Leu Gly Asn Trp Ala Arg Glu Ile Trp Ala Thr Leu
225                 230                 235                 240

TTC AAA AAG GCT ACC AGA CAA TGT AGA AGA GGT AGA ATA TGG AAA AGA   768
Phe Lys Lys Ala Thr Arg Gln Cys Arg Arg Gly Arg Ile Trp Lys Arg
                245                 250                 255
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TGG AAT GAG ACA ATA ACA GGA CCA ATA GGA TGT GCC AAT AAC ACA TGT   816
Trp Asn Glu Thr Ile Thr Gly Pro Ile Gly Cys Ala Asn Asn Thr Cys
            260                 265                 270

TAC AAT ATC TCA GTG ATA GTA CCT GAT TAT CAA TGT TAC ATA GAC AGA   864
Tyr Asn Ile Ser Val Ile Val Pro Asp Tyr Gln Cys Tyr Ile Asp Arg
        275                 280                 285

GTA GAT ACT TGG TTA CAA GGA AAA GTA AAT ATA TCA CTA TGC TTA ACA   912
Val Asp Thr Trp Leu Gln Gly Lys Val Asn Ile Ser Leu Cys Leu Thr
    290                 295                 300

GGA GGA AAA ATG TTG TAT AAT AAA GAA ACA AAA CAA TTA AGC TAT TGT   960
Gly Gly Lys Met Leu Tyr Asn Lys Glu Thr Lys Gln Leu Ser Tyr Cys
305                 310                 315                 320

ACA GAC CCA TTA CAA ATC CCA CTA ATC AAT TAT ACG TTT GGA CCT AAT  1008
Thr Asp Pro Leu Gln Ile Pro Leu Ile Asn Tyr Thr Phe Gly Pro Asn
                325                 330                 335

CAA ACA TGT ATG TGG AAC ATT TCA CAA ATT CAA GAC CCT GAA ATT CCA  1056
Gln Thr Cys Met Trp Asn Ile Ser Gln Ile Gln Asp Pro Glu Ile Pro
            340                 345                 350

AAA TGT GGA TGG TGG AAT CAA CAA GCT TAT TAT AAC AAT TGT AAA TGG  1104
Lys Cys Gly Trp Trp Asn Gln Gln Ala Tyr Tyr Asn Asn Cys Lys Trp
        355                 360                 365

GAG CGG ACT GAT GTA AAG TTT CAG TGT CAA AGA ACA CAG AGT CAG CCT  1152
Glu Arg Thr Asp Val Lys Phe Gln Cys Gln Arg Thr Gln Ser Gln Pro
    370                 375                 380

GGG TCA TGG ATT AGG GCA ATC TCG TCG TGG AAG CAA GGG AAT AGA TGG  1200
Gly Ser Trp Ile Arg Ala Ile Ser Ser Trp Lys Gln Gly Asn Arg Trp
385                 390                 395                 400

GAA TGG AGA CCA GAT TTT GAA AGT GAA AGG GTG AAA GTA TCG CTA CAA  1248
Glu Trp Arg Pro Asp Phe Glu Ser Glu Arg Val Lys Val Ser Leu Gln
                405                 410                 415

TGT AAT AGC ACA AGA AAT CTA ACC TTT GCA ATG AGA AGT TCA GGA GAT  1296
Cys Asn Ser Thr Arg Asn Leu Thr Phe Ala Met Arg Ser Ser Gly Asp
            420                 425                 430

TAT GGC GAA ATA ACG GGA GCT TGG ATA GAG TTT GGA TGT CAT AGG AAT  1344
Tyr Gly Glu Ile Thr Gly Ala Trp Ile Glu Phe Gly Cys His Arg Asn
        435                 440                 445

AAA TCA ATA CGT CAT AAT GCA GCA AGG TTT AGA ATT AGA TGT AGA TGG  1392
Lys Ser Ile Arg His Asn Ala Ala Arg Phe Arg Ile Arg Cys Arg Trp
    450                 455                 460

AAT GAA GGG GAT AAT AAC TCA CTC ATT GAT ACA TGT GGA GAA ACG CAA  1440
Asn Glu Gly Asp Asn Asn Ser Leu Ile Asp Thr Cys Gly Glu Thr Gln
465                 470                 475                 480
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AAT GTT TCA GGT GCA AAT CCT GTA GAT TGT ACC ATG TAT GCA AAT AAA  1488
Asn Val Ser Gly Ala Asn Pro Val Asp Cys Thr Met Tyr Ala Asn Lys
                485                 490                 495

ATG TAT AAT TGT TCC TTA CAA GAT GGG TTT ACT ATG AAG GTA GAT GAC  1536
Met Tyr Asn Cys Ser Leu Gln Asp Gly Phe Thr Met Lys Val Asp Asp
            500                 505                 510

CTT ATT ATG CAT TTC AAT ATG ACA AAA GCT GTA GAA ATG TAT AAC ATT  1584
Leu Ile Met His Phe Asn Met Thr Lys Ala Val Glu Met Tyr Asn Ile
        515                 520                 525

GCT GGA AAT TGG TCT TGT ATG TCT GAC TTA CCA ACA GAA TGG GGA TAT  1632
Ala Gly Asn Trp Ser Cys Met Ser Asp Leu Pro Thr Glu Trp Gly Tyr
    530                 535                 540

ATG AAT TGT AAT TGT ACC AAT GAC ACC TCT AAT AAT AAC ACT AGA AAA  1680
Met Asn Cys Asn Cys Thr Asn Asp Thr Ser Asn Asn Asn Thr Arg Lys
545                 550                 555                 560

ATG AAA TGT CCT AAG GAA AAT GGC ATC TTA AGA AAT TGG TAT AAC CCA  1728
Met Lys Cys Pro Lys Glu Asn Gly Ile Leu Arg Asn Trp Tyr Asn Pro
                565                 570                 575

GTA GCA GGA TTA AGA CAA TCC TTA GAA AAG TAT CAA GTT GTA AAA CAA  1776
Val Ala Gly Leu Arg Gln Ser Leu Glu Lys Tyr Gln Val Val Lys Gln
            580                 585                 590

CCA GAT TAC TTA CTG GTA CCA GAG GAA GTC ATG GAA TAT AAA CCT AGA  1824
Pro Asp Tyr Leu Leu Val Pro Glu Glu Val Met Glu Tyr Lys Pro Arg
        595                 600                 605

AGA AAA AGA GCA GCT ATT CAT GTT ATG TTA GCT CTT GCA ACA GTA TTA  1872
Arg Lys Arg Ala Ala Ile His Val Met Leu Ala Leu Ala Thr Val Leu
    610                 615                 620

TCT ATG GCT GGA GCA GGG ACG GGA GCT ACT GCT ATA GGG ATG GTA ACA  1920
Ser Met Ala Gly Ala Gly Thr Gly Ala Thr Ala Ile Gly Met Val Thr
625                 630                 635                 640

CAA TAT CAT CAA GTT CTG GCA ACT CAG CAA GAA GCT ATA GAA AAG GTG  1968
Gln Tyr His Gln Val Leu Ala Thr Gln Gln Glu Ala Ile Glu Lys Val
                645                 650                 655

ACT GAA GCC TTA AAG ATA ACT AAC TTA AGA TTA GTT ACA TTA GAG CAT  2016
Thr Glu Ala Leu Lys Ile Thr Asn Leu Arg Leu Val Thr Leu Glu His
            660                 665                 670

CAA GTA TTA GTA ATA GGA TTA AAA GTA GAA GCT ATG GAA AAA TTT TTA  2064
Gln Val Leu Val Ile Gly Leu Lys Val Glu Ala Met Glu Lys Phe Leu
        675                 680                 685

TAT ACA GCT TTC GCT ATG CAA GAA CTA GGA TGT AAT CAA AAT CAA TTC  2112
Tyr Thr Ala Phe Ala Met Gln Glu Leu Gly Cys Asn Gln Asn Gln Phe
    690                 695                 700
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TTC TGT AAA GTC CCT CCT GAA TTA TGG AGG AGG TAT AAT ATG ACT ATA  2160
Phe Cys Lys Val Pro Pro Glu Leu Trp Arg Arg Tyr Asn Met Thr Ile
705                 710                 715                 720

AAT CAA ACA ATA TGG AAT CAT GGA AAT ATA ACT TTA GGA GAA TGG TAT  2208
Asn Gln Thr Ile Trp Asn His Gly Asn Ile Thr Leu Gly Glu Trp Tyr
                725                 730                 735

AAC CAA ACA AAA GAT CTA CAA AAA AAG TTT TAT GGG ATA ATA ATG GAT  2256
Asn Gln Thr Lys Asp Leu Gln Lys Lys Phe Tyr Gly Ile Ile Met Asp
            740                 745                 750

ATA GAG CAA AAT AAT GTA CAA GGG AAA AAA GGG TTA CAA CAA TTA CAA  2304
Ile Glu Gln Asn Asn Val Gln Gly Lys Lys Gly Leu Gln Gln Leu Gln
        755                 760                 765

AAG TGG GAA GAT TGG GTA GGA TGG ATA GGA AAT ATA CCA CAA TAT TTA  2352
Lys Trp Glu Asp Trp Val Gly Trp Ile Gly Asn Ile Pro Gln Tyr Leu
    770                 775                 780

AAA GGA TTA TTA GGA AGT ATC GTA GGA ATA GGA TTG GGA ATC TTA TTA  2400
Lys Gly Leu Leu Gly Ser Ile Val Gly Ile Gly Leu Gly Ile Leu Leu
785                 790                 795                 800

TTG ATC TTA TGT TTA CCT ACA TTG GTT GAT TGT ATA AGA AAT TGT ATC  2448
Leu Ile Leu Cys Leu Pro Thr Leu Val Asp Cys Ile Arg Asn Cys Ile
                805                 810                 815

CAC AAG ATA CTA GGA TAC ACA GTA ATT GCA ATG CCT GAA GTA GAC GGA  2496
His Lys Ile Leu Gly Tyr Thr Val Ile Ala Met Pro Glu Val Asp Gly
            820                 825                 830

GAA GAG ATA CAA CCA CAA ATG GAA TTG AGG AGA AAT GGT AGG CAA TGT  2544
Glu Glu Ile Gln Pro Gln Met Glu Leu Arg Arg Asn Gly Arg Gln Cys
        835                 840                 845

GGC ATG TCA GAA AAA GAG GAG GAA TG                              2571
Gly Met Ser Glu Lys Glu Glu Glu
    850                 855
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 856 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Ala Glu Gly Phe Val Ala Asn Gly Gln Trp Ile Gly Pro Glu Glu
  1               5                  10                  15
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Ala Glu Glu Leu Val Asp Phe Glu Ile Ala Thr Gln Met Asn Glu Glu
            20                  25                  30

Gly Pro Leu Asn Pro Gly Ile Asn Pro Phe Arg Val Pro Gly Ile Thr
        35                  40                  45

Lys Gln Glu Lys Gln Glu Tyr Cys Ser Thr Met Gln Pro Lys Leu Gln
    50                  55                  60

Ala Leu Arg Asn Glu Ile Gln Glu Val Lys Leu Glu Glu Gly Asn Ala
65                  70                  75                  80

Gly Lys Phe Arg Arg Ala Arg Phe Leu Arg Tyr Ser Asp Glu Thr Ile
                85                  90                  95

Leu Ser Leu Ile Tyr Leu Phe Ile Gly Tyr Phe Arg Tyr Leu Val Asp
            100                 105                 110

Arg Lys Arg Phe Gly Ser Leu Arg His Asp Ile Asp Ile Glu Ala Pro
        115                 120                 125

Gln Glu Glu Cys Tyr Asn Asn Lys Glu Lys Gly Met Thr Glu Asn Ile
    130                 135                 140

Lys Tyr Gly Lys Arg Cys Leu Val Gly Thr Ala Ala Leu Tyr Leu Ile
145                 150                 155                 160

Leu Ala Ile Gly Ile Ile Ile Ile Ile Arg Thr Thr Asp Ala Gln Val
                165                 170                 175

Val Trp Arg Leu Pro Pro Leu Val Val Pro Val Glu Glu Ser Glu Ile
            180                 185                 190

Ile Phe Trp Asp Cys Trp Ala Pro Glu Glu Pro Ala Cys Gln Asp Phe
        195                 200                 205

Leu Gly Ala Met Ile His Leu Lys Ala Ser Thr Asn Ile Ser Asn Thr
    210                 215                 220

Glu Gly Pro Thr Leu Gly Asn Trp Ala Arg Glu Ile Trp Ala Thr Leu
225                 230                 235                 240

Phe Lys Lys Ala Thr Arg Gln Cys Arg Arg Gly Arg Ile Trp Lys Arg
                245                 250                 255

Trp Asn Glu Thr Ile Thr Gly Pro Ile Gly Cys Ala Asn Asn Thr Cys
            260                 265                 270

Tyr Asn Ile Ser Val Ile Val Pro Asp Tyr Gln Cys Tyr Ile Asp Arg
        275                 280                 285

Val Asp Thr Trp Leu Gln Gly Lys Val Asn Ile Ser Leu Cys Leu Thr
    290                 295                 300

Gly Gly Lys Met Leu Tyr Asn Lys Glu Thr Lys Gln Leu Ser Tyr Cys
305                 310                 315                 320
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Thr Asp Pro Leu Gln Ile Pro Leu Ile Asn Tyr Thr Phe Gly Pro Asn
325 330 335

Gln Thr Cys Met Trp Asn Ile Ser Gln Ile Gln Asp Pro Glu Ile Pro
340 345 350

Lys Cys Gly Trp Trp Asn Gln Gln Ala Tyr Tyr Asn Asn Cys Lys Trp
355 360 365

Glu Arg Thr Asp Val Lys Phe Gln Cys Gln Arg Thr Gln Ser Gln Pro
370 375 380

Gly Ser Trp Ile Arg Ala Ile Ser Ser Trp Lys Gln Gly Asn Arg Trp
385 390 395 400

Glu Trp Arg Pro Asp Phe Glu Ser Glu Arg Val Lys Val Ser Leu Gln
405 410 415

Cys Asn Ser Thr Arg Asn Leu Thr Phe Ala Met Arg Ser Ser Gly Asp
420 425 430

Tyr Gly Glu Ile Thr Gly Ala Trp Ile Glu Phe Gly Cys His Arg Asn
435 440 445

Lys Ser Ile Arg His Asn Ala Ala Arg Phe Arg Ile Arg Cys Arg Trp
450 455 460

Asn Glu Gly Asp Asn Asn Ser Leu Ile Asp Thr Cys Gly Glu Thr Gln
465 470 475 480

Asn Val Ser Gly Ala Asn Pro Val Asp Cys Thr Met Tyr Ala Asn Lys
485 490 495

Met Tyr Asn Cys Ser Leu Gln Asp Gly Phe Thr Met Lys Val Asp Asp
500 505 510

Leu Ile Met His Phe Asn Met Thr Lys Ala Val Glu Met Tyr Asn Ile
515 520 525

Ala Gly Asn Trp Ser Cys Met Ser Asp Leu Pro Thr Glu Trp Gly Tyr
530 535 540

Met Asn Cys Asn Cys Thr Asn Asp Thr Ser Asn Asn Asn Thr Arg Lys
545 550 555 560

Met Lys Cys Pro Lys Glu Asn Gly Ile Leu Arg Asn Trp Tyr Asn Pro
565 570 575

Val Ala Gly Leu Arg Gln Ser Leu Glu Lys Tyr Gln Val Val Lys Gln
580 585 590

Pro Asp Tyr Leu Leu Val Pro Glu Glu Val Met Glu Tyr Lys Pro Arg
595 600 605

Arg Lys Arg Ala Ala Ile His Val Met Leu Ala Leu Ala Thr Val Leu
610 615 620

Ser Met Ala Gly Ala Gly Thr Gly Ala Thr Ala Ile Gly Met Val Thr
625 630 635 640

Gln Tyr His Gln Val Leu Ala Thr Gln Gln Glu Ala Ile Glu Lys Val
645 650 655

Thr Glu Ala Leu Lys Ile Thr Asn Leu Arg Leu Val Thr Leu Glu His
660 665 670

Gln Val Leu Val Ile Gly Leu Lys Val Glu Ala Met Glu Lys Phe Leu
675 680 685

Tyr Thr Ala Phe Ala Met Gln Glu Leu Gly Cys Asn Gln Asn Gln Phe
690 695 700

Phe Cys Lys Val Pro Pro Glu Leu Trp Arg Arg Tyr Asn Met Thr Ile
705 710 715 720

Asn Gln Thr Ile Trp Asn His Gly Asn Ile Thr Leu Gly Glu Trp Tyr
725 730 735

Asn Gln Thr Lys Asp Leu Gln Lys Lys Phe Tyr Gly Ile Ile Met Asp
740 745 750

Ile Glu Gln Asn Asn Val Gln Gly Lys Lys Gly Leu Gln Gln Leu Gln
755 760 765

Lys Trp Glu Asp Trp Val Gly Trp Ile Gly Asn Ile Pro Gln Tyr Leu
770 775 780

Lys Gly Leu Leu Gly Ser Ile Val Gly Ile Gly Leu Gly Ile Leu Leu
785 790 795 800

Leu Ile Leu Cys Leu Pro Thr Leu Val Asp Cys Ile Arg Asn Cys Ile
805 810 815

His Lys Ile Leu Gly Tyr Thr Val Ile Ala Met Pro Glu Val Asp Gly
820 825 830

Glu Glu Ile Gln Pro Gln Met Glu Leu Arg Arg Asn Gly Arg Gln Cys
835 840 845

Gly Met Ser Glu Lys Glu Glu Glu
850 855

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 255 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(B) STRAIN: FIV-113

(ix) FEATURE:
(A) NAME/KEY: CF
(B) LOCATION: 1081..1335

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GCT TAT TAT AAC AAT TGT AAA TGG GAG CGG ACT GAT GTA AAG TTT CAG     48
Ala Tyr Tyr Asn Asn Cys Lys Trp Glu Arg Thr Asp Val Lys Phe Gln
 1               5                  10                  15

TGT CAA AGA ACA CAG AGT CAG CCT GGG TCA TGG ATT AGG GCA ATC TCG     96
Cys Gln Arg Thr Gln Ser Gln Pro Gly Ser Trp Ile Arg Ala Ile Ser
            20                  25                  30

TCG TGG AAG CAA GGG AAT AGA TGG GAA TGG AGA CCA GAT TTT GAA AGT    144
Ser Trp Lys Gln Gly Asn Arg Trp Glu Trp Arg Pro Asp Phe Glu Ser
        35                  40                  45

GAA AGG GTG AAA GTA TCG CTA CAA TGT AAT AGC ACA AGA AAT CTA ACC    192
Glu Arg Val Lys Val Ser Leu Gln Cys Asn Ser Thr Arg Asn Leu Thr
    50                  55                  60

TTT GCA ATG AGA AGT TCA GGA GAT TAT GGC GAA ATA ACG GGA GCT TGG    240
Phe Ala Met Arg Ser Ser Gly Asp Tyr Gly Glu Ile Thr Gly Ala Trp
65                  70                  75                  80

ATA GAG TTT GGA TGT                                            255
Ile Glu Phe Gly Cys
                85
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ala Tyr Tyr Asn Asn Cys Lys Trp Glu Arg Thr Asp Val Lys Phe Gln
 1               5                  10                  15

Cys Gln Arg Thr Gln Ser Gln Pro Gly Ser Trp Ile Arg Ala Ile Ser
            20                  25                  30

Ser Trp Lys Gln Gly Asn Arg Trp Glu Trp Arg Pro Asp Phe Glu Ser
        35                  40                  45

Glu Arg Val Lys Val Ser Leu Gln Cys Asn Ser Thr Arg Asn Leu Thr
    50                  55                  60

Phe Ala Met Arg Ser Ser Gly Asp Tyr Gly Glu Ile Thr Gly Ala Trp
65                  70                  75                  80

Ile Glu Phe Gly Cys
                85
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: AISSWKQGNRWE
(B) LOCATION: 390..401

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Ala Ile Ser Ser Trp Lys Gln Gly Asn Arg Trp Glu
1               5                  10
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: KQGNRWEWRPD
(B) LOCATION: 395..405

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Lys Gln Gly Asn Arg Trp Glu Trp Arg Pro Asp
1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: WEWRPDFESERV
(B) LOCATION: 400..411

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Trp Glu Trp Arg Pro Asp Phe Glu Ser Glu Arg Val
1 5 10

CLAIMS

1) Vaccine comprising a polypeptide fragment of the Feline Immuno-deficiency Virus surface protein, characterised in that said polypeptide fragment comprises an amino acid sequence of the Central Fragment (SEQ ID NO: 4.) or a portion thereof, or an epitope located in the central fragment, said epitope being capable of inducing antibodies that competitively inhibit binding of the neutralising monoclonal antibody from hybridoma 1E1EB4-93030567 as deposited with the ECACC to native surface protein, said fragment being capable of inducing neutralising antibodies against Feline Immuno-deficiency Virus.

2) Vaccine according to claim 1, characterised in that the polypeptide fragment is a portion of the Central Fragment and that said portion comprises at least an epitope located in between amino acid 390 and amino acid 412.

3) Vaccine according to claim 2, characterised in that the portion is selected from the group consisting of SEQ ID NO: 5, 6 and 7.

4) Immunogen comprising a polypeptide fragment of the Feline Immuno-deficiency Virus surface protein, characterised in that said polypeptide fragment comprises: - an amino acid sequence of the Central Fragment (SEQ ID NO: 4.) or a portion thereof preferably comprising at least an epitope located in between amino acid 390 and amino acid 412, more preferably selected from the group consisting of SEQ ID NO: 5, 6 and 7;

- or an epitope located in the central fragment, said epitope being capable of inducing antibodies that competitively inhibit binding of the neutralising monoclonal antibody from hybridoma 1E1EB4-93030567 as deposited with the ECACC to native surface protein; said fragment being capable of inducing neutralising antibodies against Feline Immuno-deficiency Virus.

5) Immunogen according to claim 4, characterised in that the carrier is selected from the group of carriers consisting of surface active compounds, sugars and proteins.

6) Nucleic acid sequence encoding a polypeptide fragment of the Feline Immuno-deficiency Virus surface protein, characterised in that said polypeptide fragment comprises: - an amino acid sequence of the Central Fragment (SEQ ID NO: 4.) or a portion thereof preferably comprising at least an epitope located in between amino acid 390 and amino acid 412, more preferably selected from the group consisting of SEQ ID NO: 5, 6 and 7;
- or an epitope located in the central fragment, said epitope being capable of inducing antibodies that competitively inhibit binding of the neutralising monoclonal antibody from hybridoma 1E1EB4-93030567 as deposited with the ECACC to native surface protein; said fragment being capable of inducing neutralising antibodies against Feline Immuno-deficiency Virus.

7) Nucleic acid sequence according to claim 6, characterised in that it comprises at least part of the nucleic acid sequence shown in SEQ ID NO: 3.

8) Recombinant nucleic acid molecule comprising a nucleic acid sequence according to claims 6 or 7, under the control of regulating sequences enabling expression of a protein encoded by said nucleic acid sequence.

9) Virus vector containing a nucleic acid molecule according to claims 6-7, or a recombinant nucleic acid molecule according to claim 8.

10) Host cell containing a nucleotide sequence according to claims 6-7, a recombinant nucleic acid sequence according to claim 8 or a vector virus according to claim 9.

11) Vaccine for the protection of cats against Feline Immune- deficiency Virus infections, comprising a nucleic acid sequence according to claims 6-7, a recombinant nucleic acid sequence according to claim 8, a virus vector according to claim 9, a host cell according to claim 10 or an immunogen according to claims 4-5.

12) Monoclonal antibody reactive with a polypeptide fragment of the Feline Immuno-deficiency Virus surface protein, characterised in that said polypeptide fragment comprises:
- an amino acid sequence of the Central Fragment (SEQ ID NO: 4.) or a portion thereof preferably comprising at least an epitope located in between amino acid 390 and amino acid 412, more preferably selected from the group consisting of SEQ ID NO: 5, 6 and 7;
- or an epitope located in the central fragment, said epitope being capable of inducing antibodies that competitively inhibit binding of the neutralising monoclonal antibody from hybridoma 1E1EB4-93030567 as deposited with the ECACC to native surface protein;

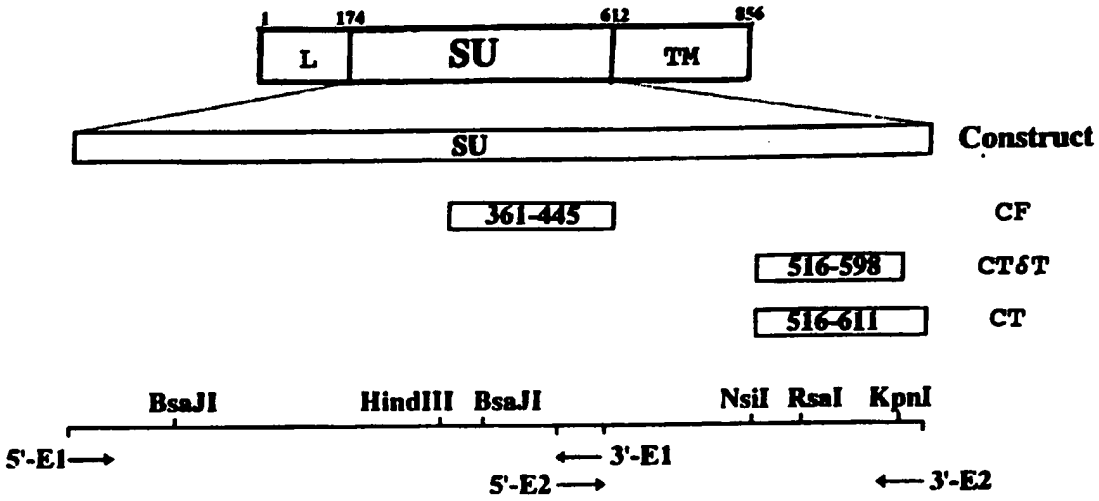
said fragment being capable of inducing neutralising antibodies against Feline Immuno-deficiency Virus.

13) Monoclonal antibody according to claim 12, characterised in that it is produced by the hybridoma 1E1EB4-93030567 deposited with the ECACC.

14) Use of an immunogen according to claim 4 or 5 or a polypeptide fragment of the Feline Immuno-deficiency Virus surface protein which comprises:
- an amino acid sequence of the Central Fragment (SEQ ID NO: 4.) or a portion thereof preferably comprising at least an epitope located in between amino acid 390 and amino acid 412, more preferably selected from the group consisting of SEQ ID NO: 5, 6 and 7;
- or which comprises an epitope located in the central fragment, said epitope being capable of inducing antibodies that competitively inhibit binding of the neutralising monoclonal antibody from hybridoma 1E1EB4-93030567 as deposited with the ECACC to native surface protein;
said fragment being capable of inducing neutralising antibodies against Feline Immuno-deficiency Virus;

for the preparation of a vaccine for the prophylaxis of Feline Immuno-deficiency Virus infection.

Figure 1.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HindIII
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NsiI
RsaI
KpnI
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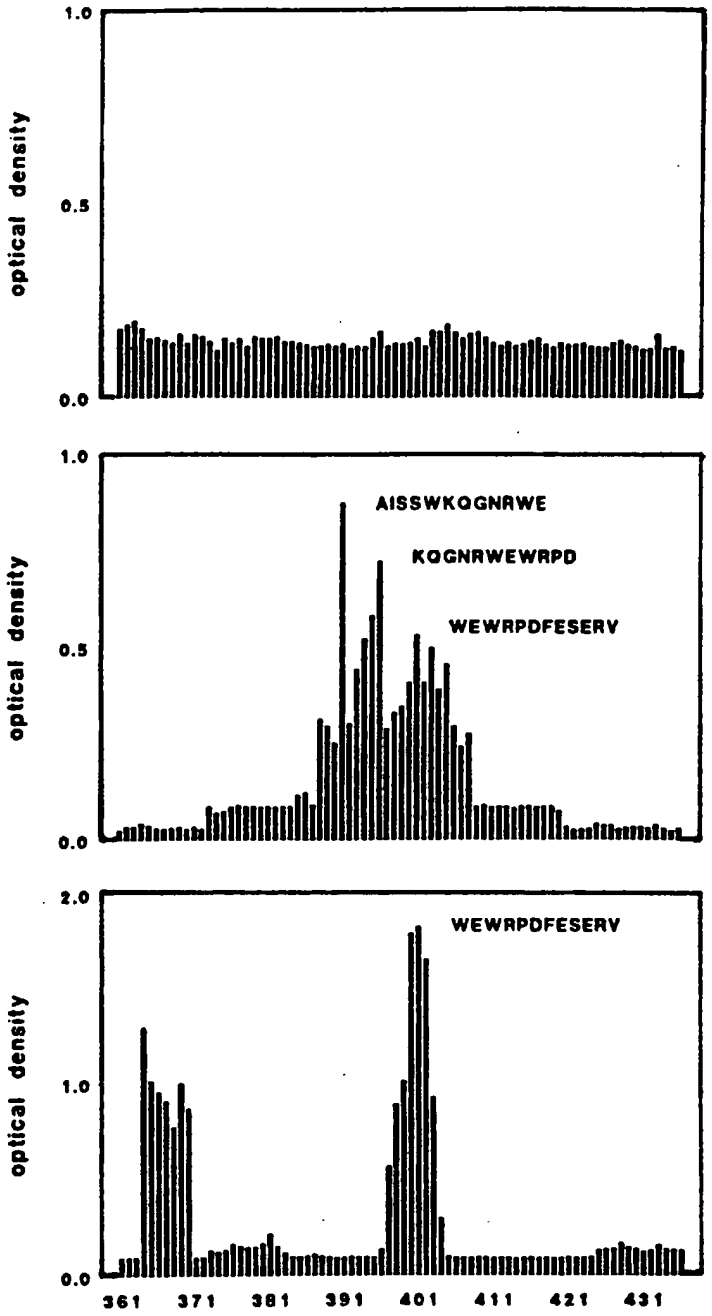
3'-E1
5'-E2
3'-E2

Figure 2: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0.5
0.0
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optical density
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0.0
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KQGNRWEWRPD
WEWRPDFESERV
2B
optical density
2.0
1.0
0.0
WEWRPDFESERV
361
371
381
391
401
411
421
431
2C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 94/00812

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/49 C07K7/06 C07K7/08 C07K15/00 A61K39/21
C12P21/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K C12N A61K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. VIROL. vol. 67, no. 2 , 1993 pages 664 - 672 G. PANCINO ET AL. 'B epitopes and selection pressures in feline immunodeficiency virus envelope glycoproteins' see page 667, column 2, line 1 - line 5; figure 1; table 1 see page 669, column 2, line 5 - page 670, column 1, line 7 --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 1994	- 1. 08. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Skelly, J

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 94/00812

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VIROLOGY vol. 192 , 1993 pages 659 - 662 G. PANCINO ET AL. 'Structure and variations of feline immunodeficiency virus envelope glycoproteins' see page 662, column 1, line 7 - line 26	1-10
A	VIRUS RESEARCH vol. 21 , 1991 pages 53 - 63 S. MORIKAWA 'Identification of conserved and variable regions in the envelope glycoprotein sequences of two feline immunodeficiency viruses isolated in Zurich Switzerland' see page 58	1-10
A	PROC. NATL. ACAD. SCI. USA vol. 86 , 1989 pages 5743 - 5747 R. TALBOTT ET AL. 'Nucleotide sequence and genomic organisation of feline immunodeficiency virus'	
A	WO,A,92 09632 (CENTR NATIONAL DE LA RECHERCHE SCIENTIFIQUE) 11 June 1992	
X,P	EP,A,0 577 458 (CENTR NATIONAL DE LA RECHERCHE SCIENTIFIQUE) 5 January 1994 see page 26 - page 27; claims 27,28,36,37	1-14

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/EP 94/00812

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9209632	11-06-92	FR-A-	2669338	22-05-92
		EP-A-	0564477	13-10-93
EP-A-0577458	05-01-94	FR-A-	2692279	17-12-93
		FR-A-	2692269	17-12-93
		FR-A-	2692270	17-12-93